

### Subpart D—Molecular Examination Procedures

SOURCE: 72 FR 1425, Jan. 12, 2007, unless otherwise noted.

**§ 147.30 Laboratory procedure recommended for the polymerase chain reaction (PCR) test for *Mycoplasma gallisepticum* and *M. synoviae*.**

(a) *DNA isolation.* Isolate DNA from 1 mL of eluate from tracheal swabs in PBS or 1 mL of broth culture by a non-phenolic procedure. Centrifuge samples at 14,000 x g for 5 to 10 minutes. Decant

supernatant and wash the pellet with 1 mL of PBS. Centrifuge as above and resuspend the pellet in 25–50 µl of 0.1 percent DEP (Diethyl Pyrocarbonate; Sigma) water. Boil at 120 °C for 10 minutes followed by 10 minutes incubation at 4 °C. Centrifuge as above and transfer the supernatant DNA to a nuclease-free tube. Estimate the DNA concentration and purity by spectrophotometric reading at 260 nm and 280 nm.

(b) *Primer selection.* (1) *M. gallisepticum.* The primer for *M. gallisepticum* should consist of the following sequences:

MG-F	5' GAG CTA ATC TGT AAA GTT GGT C
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MG-R	5' GCT TCC TTG CGG TTA GCA AC
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(2) *M. synoviae.* The primer for *M. synoviae* should consist of the following sequences:

MS-F	5' GAG AAG CAA AAT AGT GAT ATC A
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MS-R	5' CAG TCG TCT CCG AAG TTA ACA A
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(c) *Polymerase chain reaction.* (1) Treat each sample (100 to 2000 ng/5 µl) with one of the following 45 µl PCR cocktails:

(i) 5 µl 10x PCR buffer, 1 µl dNTP (10 mM), 1 µl of Reverse primer (50 µM), 1 µl of Forward primer (50 µM), 4 µl MgCl<sub>2</sub> (25 mM), 1 µl taq-polymerase (5 U), 32 µl DEP water.

(ii) 18 µl water, 25 µl PCR mix (Promega), 1 µl Reverse primer (50 µM), 1 µl Forward primer (50 µM).

(2) Perform DNA amplification in a Perkin-Elmer 9600 thermocycler or in a Hybaid PCR Express thermocycler.<sup>24</sup> The optimized PCR program is as follows:

Temperature ( °C)	Duration	Cycles
94 .....	30 seconds .....	30–40.
55 .....	30 seconds .....	30–40.
72 .....	1 minute .....	30–40.
72 .....	5 minutes .....	1 (final extension).

<sup>24</sup>Trade names are used in these procedures solely for the purpose of providing specific information. Mention of a trade name does not constitute a guarantee or warranty of

the product by the U.S. Department of Agriculture or an endorsement over other products not mentioned.

(d) *Electrophoresis*. Mix PCR products (5 to 10 µl) with 2 µl loading buffer (Sigma) and electrophorese on a 2 percent agarose gel containing 0.5 µg/mL ethidium bromide in TAE buffer (40 mM tris; 2 mM EDTA; pH 8.0 with glacial acetic acid) for 30 minutes at 80 V. *M. gallisepticum* (185 bp) and *M. synoviae* (214 bp) amplicons can be visualized under an ultraviolet transilluminator along with the PCR marker (50 to 2000 bp; Sigma).

### Subpart E—Procedure for Changing National Poultry Improvement Plan

#### § 147.41 Definitions.

Except where the context otherwise requires, for the purposes of this subpart the following terms shall be construed, respectively, to mean:

*Department*. The U.S. Department of Agriculture.

*Egg type chickens*. Chickens bred for the primary purpose of producing eggs for human consumption.

*Exhibition Poultry*. Domesticated fowl which are bred for the combined purposes of meat or egg production and competitive showing.

*Game birds*. Domesticated fowl, such as pheasants, partridge, quail, grouse, and guineas, but not doves and pigeons.

*Meat type chickens*. Chickens bred for the primary purpose of producing meat.

*Plan Conference*. A meeting convened for the purpose of recommending changes in the provisions of the Plan.

*Plan or NPIP*. The National Poultry Improvement Plan.

*Service*. The Animal and Plant Health Inspection Service, Veterinary Services, of the Department.

*State*. Any State, the District of Columbia, or Puerto Rico.

*Waterfowl*. Domesticated fowl that normally swim, such as ducks and geese.

[36 FR 23121, Dec. 3, 1971, as amended at 38 FR 3038, Feb. 1, 1973. Redesignated at 44 FR 61586, Oct. 26, 1979; 59 FR 12805, Mar. 18, 1994]

#### § 147.42 General.

Changes in this subchapter shall be made in accordance with the procedure described in this subpart: *Provided*,

That the Department reserves the right to make changes in this subchapter without observance of such procedure when such action is deemed necessary in the public interest.

#### § 147.43 General Conference Committee.

(a) The General Conference Committee Chairperson and the Vice Chairperson shall be elected by the members of the General Conference Committee. A representative of the Animal and Plant Health Inspection Service will serve as Executive Secretary and will provide the necessary staff support for the General Conference Committee. The General Conference Committee shall consist of one member-at-large who is a participant in the National Poultry Improvement Plan and one member to be elected, as provided in paragraph (b) of this section, from each of the following regions:

(1) North Atlantic: Maine, New Hampshire, Vermont, Massachusetts, Rhode Island, Connecticut, New York, New Jersey, and Pennsylvania.

(2) East North Central: Ohio, Indiana, Illinois, Michigan, and Wisconsin.

(3) West North Central: Minnesota, Iowa, Missouri, North Dakota, South Dakota, Nebraska, and Kansas.

(4) South Atlantic: Delaware, District of Columbia, Maryland, Virginia, West Virginia, North Carolina, South Carolina, Georgia, Florida, and Puerto Rico.

(5) South Central: Kentucky, Tennessee, Alabama, Mississippi, Arkansas, Louisiana, Oklahoma, and Texas.

(6) Western: Montana, Idaho, Wyoming, Colorado, New Mexico, Arizona, Utah, Nevada, Washington, Oregon, California, Alaska, and Hawaii.

(b) The regional committee members and their alternates will be elected by the official delegates of their respective regions, and the member-at-large will be elected by all official delegates. There must be at least two nominees for each position, the voting will be by secret ballot, and the results will be recorded. At least one nominee from each region must be from an underrepresented group (minorities, women, or persons with disabilities). The process for soliciting nominations for regional committee members will include, but